

Feeding strategy assessment through fatty acid profiles in muscles of adult sea lampreys from the western Iberian coast

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SUMMARY: The fatty acid signature of sea lamprey *Petromyzon marinus* (L.) muscle was used as a tool to detect feeding strategies used during the parasitic marine trophic phase of the species. Adult sea lampreys were collected near the mouth of six Portuguese rivers (Minho, Lima, Douro, Vouga, Mondego and Tagus) and muscle fatty acid profile was characterized. The analysis of fatty acid composition of muscle neutral lipids showed the formation of two groups, indicating that two feeding strategies may have been used by sea lampreys during the parasitic phase, based on the availability of ω -3 and ω -6 *PUFA* and on evidence of phytoplankton/zooplankton and bacterial detritus contribution in the sea lamprey host preferences. Two distinct lipid profiles were observed, probably related to two different trophic approaches, one typical of a top predator of a marine food web with a planktonic support, and the other much more diverse, including the same planktonic markers, together with biochemical clues that probably resulted from a parasitic phase that directly targeted fish that consumed detritus and benthic algae and/or fish from a food web with a detritivorous base.

Keywords: *Petromyzon marinus*, feeding strategies, marine phase, fatty acids, Portugal.

RESUMEN: EVALUACIÓN DE ESTRATEGIAS DE ALIMENTACIÓN MEDIANTE LA UTILIZACIÓN DE PERFILES DE ÁCIDOS GRASOS EN LOS MÚSCULOS DE LAMPREA MARINA ADULTA DE LA COSTA OCCIDENTAL IBÉRICA. – Las composiciones de los ácidos grasos en los músculos de lamprea marina *Petromyzon marinus* (L.) fueron usadas como herramienta para detectar la estrategia de alimentación de la especie durante la fase marina de alimentación parasítica. Se recolectaron individuos adultos cerca de la desembocadura de las cuencas hidrográficas de seis ríos portugueses (Miño, Lima, Douro, Vouga, Mondego y Tago) y se caracterizaron los perfiles de los contenidos en ácidos grasos en el músculo. El análisis de la composición en ácidos grasos de los lípidos neutros del músculo muestra la formación de dos grupos, lo que indica que las lampreas en su fase parasítica pueden usar dos estrategias de alimentación basadas en la disponibilidad de ω -3 y ω -6 *PUFA* y en la contribución de fitoplancton/zooplancton y detritos bacterianos en las preferencias alimenticias de los huéspedes de la lamprea marina. Se observaron dos perfiles lipídicos distintos que, probablemente, están relacionados con dos aproximaciones tróficas diferentes, una típica de un predador superior de cadena trófica marina con una base planctónica y otra, mucho más variada, que incluye los mismos marcadores planctónicos junto a indicios bioquímicos que probablemente son el resultado de una fase parasítica de peces consumidores de detritus y algas bentónicas, y/o la depredación de peces de cadena trófica con una base detritívora.

Palabras clave: *Petromyzon marinus*, estrategias de alimentación, fase marina, ácidos grasos, Portugal.

INTRODUCTION

The life cycle of the sea lamprey *Petromyzon marinus* (L.) contains a microphagous larval phase, which is spent in freshwater (Potter 1980). After four to seven years, a metamorphosis period occurs which transforms the microphagous filter-feeding larvae into pelagic juveniles. This is a non-trophic period during which lampreys utilize the lipid reserves accumulated in the larval phase (Bird and Potter 1983). The pelagic juveniles then migrate downstream to the sea, where they begin the parasitic stage of their life cycle, feeding primarily on blood and muscle tissue of marine fish for as much as 13 to 28 months (Beamish 1980, Silva *et al.* 2013a). This marine phase is accompanied by the deposition of large amounts of lipids, particularly in the musculature, which act as the primary energy source during the upstream migration (Sheridan 1988, Bird *et al.* 1993). At the end of this oceanic phase the adults cease feeding and enter rivers to migrate upstream, where they spawn and die (Larsen 1980).

Little information on the feeding ecology of sea lampreys in the marine environment is available because few specimens have been captured in the ocean and reports of fish with attachment scars are relatively scarce (Farmer 1980). Nonetheless, sea lampreys seem to prefer fish that have smaller, less protective scales and are thus easier to feed on and fish with benthopelagic or pelagic modes of life. Marine organisms reported to have been preyed on by sea lamprey include bony fish, elasmobranchs and cetaceans (Beamish 1980, Halliday 1991, Nichols and Hamilton 2004). This paucity of information concerning the ecology of adult sea lamprey during the parasitic marine stage, including the preferential host species, is shared by most of the remaining species of anadromous parasitic lampreys (Renaud *et al.* 2009).

It has been reported that fatty acids can be used as biological markers and general diet indicators in marine ecosystems (Sargent *et al.* 1987). An important aspect of fatty acid trophic markers is that fatty acid composition of an animal represents the time-integrated dietary intake and, because lipid metabolism and storage in animals are organ-specific, fatty acid markers should be extracted from individual tissues or body parts (Napolitano 1999). The fatty acid profile of adipose tissue triacylglycerols is particularly useful as a trophic marker because fatty acids accumulate over time, representing an integration of dietary intake over days, weeks or even months (Hendersen and Sargent 1981, Leger *et al.* 1981, Iverson 2009), whereas examinations of gut content of an animal provide only information of the last ingesta (Napolitano 1999).

More recently, fatty acids have been used to purposely identify food web relationships because of their biological specificity and because, although organisms are able to biosynthesize and modify fatty acid chains, these processes are phylogenetically limited (Iverson

2009). Previous studies have used fatty acids as biomarkers for bacteria (Rajendran *et al.* 1993), diatoms (Parrish *et al.* 2000), zooplankton and crustacean zooplankton (Falk-Petersen *et al.* 2002, Brett *et al.* 2009), macroalgae (Johns *et al.* 1979, Khotimchenko and Vaskovsky 1990) and vascular plants (Wannigama *et al.* 1981).

In a previous study, the authors found that the fatty acid profile of the neutral and polar lipids of sea lamprey muscle at the beginning of upriver migration show differences consistent with the river basin of origin (Pinela *et al.* 2009), thus raising some questions concerning the meaning of this discovery. A subsequent study concluded that muscle fatty acid profiles of neutral lipids could be used as a signature of sea lamprey diets during their marine trophic phase (Lança *et al.* 2011). Bearing this in mind, the main objective of this study was to assess the feeding strategy of sea lamprey during the marine trophic phase off the Portuguese coast by analysing the fatty acid composition of muscle neutral lipids.

Some of the sea lampreys that enter Portuguese rivers during their spawning migration attain 2.0 kg (unpublished data), which means that they must have been targeting large preys. Besides the scientific interest of revealing part of the feeding ecology of this species, there is also some concern regarding the management and conservation of this important resource for inland Portuguese fisheries. In fact, it is important to know as much as possible about the ecology of their adult trophic phase, in particular with regard to the range of dispersion in the marine environment. This knowledge may provide the bases for the spatial definition of future conservation units, which may range from a single basin level to a more regional, or even international, geographical scale. As a result of our constant demand for new fishing grounds, particularly in the deep ocean, measures of this type will be necessary in the near future in order to avoid the collapse of this fishery due to a potential overexploitation of their preferential hosts by commercial fisheries.

MATERIALS AND METHODS

Samples and sampling sites

Sampling occurred at the peak of the sea lamprey spawning migration period (March 2008) in the basins of six Portuguese rivers (Minho, Lima, Douro, Vouga, Mondego and Tagus). The Minho, Lima and Douro river basins are located in the northwest area of Portugal, whereas the Vouga, Mondego and Tagus river basins are located in the central region of the country. Sea lampreys were collected with trammel nets by professional fishermen near the mouth of each river, except in the River Tagus, where individuals were caught 67 km upstream from the river mouth (Fig. 1). A total of 115 sea lampreys (*ca.* 19 individuals per river basin) were sampled and transported alive to the laboratory

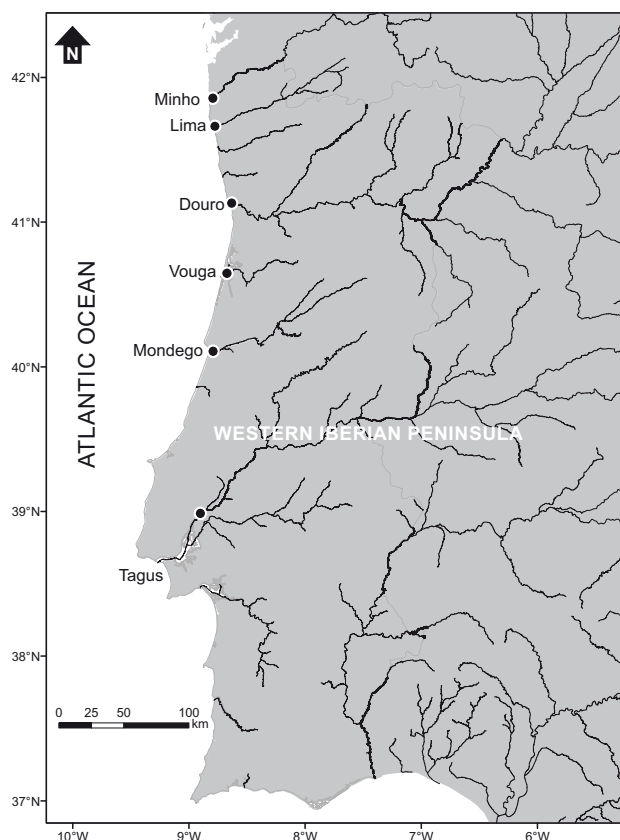


FIG. 1. – Location of the sampling sites of sea lamprey captures (*Petromyzon marinus*, L.) (●) in the Rivers Minho, Lima, Douro, Vouga, Mondego and Tagus (Portugal).

in a 0.4-m³ capacity tank equipped with an appropriate life-support system including aeration, water quality maintenance system and temperature control. Transportation time between the sampling site and the laboratory where the individuals were processed ranged from 3 to 5 hours.

Tissue preparation and collection

Data on body total mass (M_T , nearest g) and total length (L_T , nearest mm – length between the beginning of the oral disk to the end of the caudal fin) was registered for each sea lamprey. All trunk muscle between the posterior edge of the last branchial opening and the anterior edge of the cloacal slit on the left flank of the animal were collected, washed with physiological saline solution and immediately stored in liquid nitrogen (–196°C) until processing in the laboratory.

Muscle lipid extraction and fatty acid analysis

Muscle lipids were obtained using accelerated solvent extraction, an automated procedure that makes use of pressurized solvents at high temperatures. To prepare for extraction, the tissue samples were removed from liquid nitrogen, weighed and lyophilized until constant mass to determine the percentage of water loss

(W_M). Aliquots of a 1-g portion of lyophilized muscle samples were weighed on an analytical balance (Mettler AT201; Greifensee, Switzerland) and their masses were recorded to the nearest 0.01 mg. Aliquots were then pulverized in an aluminum mortar with a stainless steel pestle, both cooled in liquid nitrogen. The tissue powder was combined with 1 g of hydromatrix drying agent (Diatomaceous Earth, hydromatrix Varian, P/N 049458), the hydromatrix mixture (tissue powder plus hydromatrix drying agent) was transferred to an 11-mL stainless steel extraction cell fitted with two cellulose filters, and additional hydromatrix drying agent was added to fill the cell. Nonadecanoic acid (C19:0) was added as a surrogate to assess the performance of all subsequent sample preparation steps because the tissues under study were known to lack the corresponding fatty acid. The total lipid sample was then extracted with a mixture of 60% chloroform and 40% methanol (Merck; Darmstadt, Germany) at 100°C and 13.8 MPa. Both extraction solvents were residue-analysis grade and were treated with 100 mg L⁻¹ BHT (3,5-Di-tert-butyl-4-hydroxytoluene, Merck; Darmstadt, Germany) as an antioxidant. Two static extraction cycles were carried out during a five-minute period each. The crude extract was then concentrated under a stream of nitrogen and vacuum using a TurboVap apparatus (Zymark; Hopkinton, MA) set at a bath temperature of 50°C and the dry mass of recovered material was measured to the nearest 0.01 mg to determine muscle total lipids (TL_M). Then, each sample was reconstituted in 20-30 volumes of ice-cold acetone to separate neutral lipids (NL) from polar lipids (PL). The samples were mixed by vortexing for one minute and left on ice for 60 minutes, and then the NL plus PL fraction was centrifuged at 3000 g for 30 minutes. The upper phase, corresponding to NL, was removed as completely as possible, evaporated under nitrogen and vacuum and measured to the nearest 0.01 mg to determine muscle neutral lipids (NL_M). The NL phase was saponified in methanolic NaOH 0.5 N at 70°C for 15 min. For transesterification, samples were treated with 1 mL of newly opened BF₃ in methanol (10 g BF₃/l CH₃OH, Merk-Schuchardt, Germany) in order to give fatty acid methyl esters (FAME) according to the procedure of Morrison and Smith (1964).

The FAME were analysed by liquid-gas chromatography in a Hewlett Packard HP 6890 Series GC System equipped with a split-splitless injector, an autosampler, a flame-ionization detector (FID), an Omega-wax 320 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness, Supelco, Bellefonte, PA) and HPChem software (2002). The chromatographic conditions were as follows: oven temperature ranged from 140° to 240°C with a ramping rate of 4°C min⁻¹; injection port temperature was 250°C and FID was 270°C. The carrier gas was (He) and flow rate was 1.2 mL min⁻¹. The resulting peak areas were corrected by theoretical relative FID response factors (Ackman 2002). The FAME were identified by comparison of their retention times with known standards (37-com-

TABLE 1. – Mean (\pm standard deviation) total length (L_T), total mass (M_T), muscle total lipids (TL_M), muscle neutral lipids (NL_M) and muscle water content (W_M) of sea lampreys (*Petromyzon marinus*) from Cluster Groups I and II.

	L_T , mm	M_T , g	TL_M , mg g ⁻¹	NL_M , mg g ⁻¹	W_M , %
Group I (N=59)	864.9 \pm 61.0	1222.9 \pm 194.0	327.4 \pm 90.0	243.5 \pm 50.9	67.1 \pm 4.7
Group II (N=51)	868.6 \pm 48.9	1210 \pm 193.9	404.5 \pm 126.9	251.6 \pm 43.2	67 \pm 3.0

NL, neutral lipid content expressed as mg g⁻¹ dry tissue; TL, total lipid content expressed in mg of TL g⁻¹ dry tissue.

ponent FAME mix, Supelco plus C22:5 ω 3 fatty acid methyl ester) chromatographed in identical gas chromatography conditions. For each sample, the relative fatty acid composition was quantified and data are presented as the weight percentage for fatty acid composition. Fatty acids were designated according to the nomenclature of the International Union of Pure and Applied Chemistry (IUPAC) for carbon chain length: number of double bonds and position of the double bond closest to the omega carbon.

The frequency of occurrence of a certain fatty acid was calculated as the percentage of sea lampreys from a specific group (geographical or other) that had that fatty acid in their muscle samples.

Data analysis and interpretation

The SPSS statistical package for Windows (version 18.0) and Primer 6.0 were used for data processing and statistical analysis. Data were arcsine transformed to meet assumptions of normality, independence and homoscedasticity. The integrated chromatogram values for each fatty acid were expressed as a percentage of the total sum of fatty acids identified in order to eliminate concentration effects.

Multivariate analysis of variance (MANOVA) was used to see the main and interaction effect of the categorical variable (gender) on M_T , L_T , muscle total lipids (TL_M), muscle neutral lipids (NL_M) and muscle water content (W_M). The Spearman rank correlation was used to analyse the relationship between muscle total lipids and muscle water content.

The cluster analysis is a numerical technique for defining groups of related samples based on high similarity coefficients computed between each pair of samples, which are then clustered. In most clustering procedures the nucleus of clusters (centroid) is formed by joining the samples with the greatest similarity and gradually admitting more samples as the similarity coefficient is lowered (Kaiser and Esterby 1991). A group average cluster analysis was performed using Spearman rank correlation to investigate the existence of groups of samples with similar muscle NL fatty acid profiles.

A distance-based permutational multivariate analysis of variance (PERMANOVA) based on the previously obtained Spearman rank correlation resemblance matrix was used to compare fatty acid muscle NL profile among the groups that corresponded to clusters obtained by the cluster analysis. The analysis was

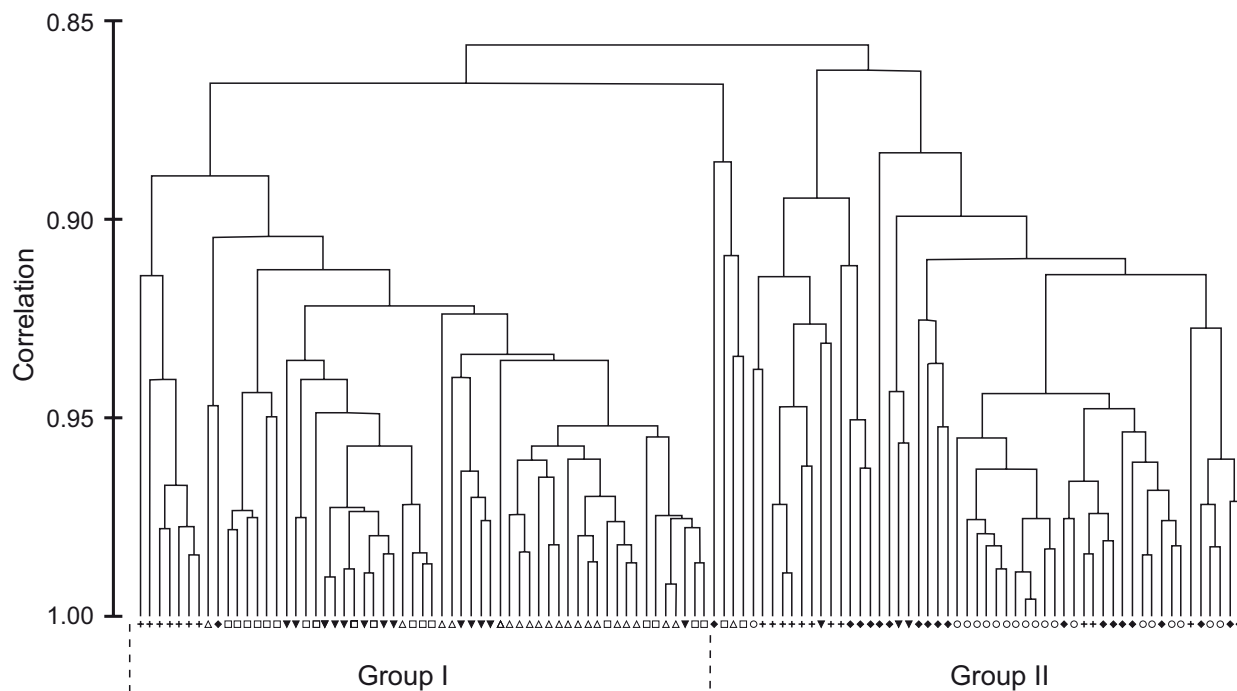


FIG. 2. – Cluster of the fatty acid profiles of sea lampreys (*Petromyzon marinus*) from the six Portuguese basins. (▼) Minho; (+) Lima; (○) Douro; (△) Vouga; (◆) Mondego; (□) Tagus.

done using 999 random permutations of the appropriate units (Anderson and ter Braak 2003). The data set comprised 110 observations × 31 variables (i.e. fatty acids) and the design included one factor: group (two levels fixed). A permutation test was used to assess the significance of the overall fatty acids among groups. Since the factor group was seen to be significant, it was further analysed through a pairwise comparison test. A SIMPER test (similar percentages) was used to determine the specific variables that contributed to overall differences, i.e. which fatty acids had more influence on dissimilarities among groups (Warwick *et al.* 1990, Clarke 1993).

RESULTS

Multivariate tests for individual effect overall revealed that gender (GLM test, $F=2.156$; $df=5$; $P=0.121$) had no significant effect on total length (L_T) and total mass (M_T). For biochemical parameters W_M , TL_M and

NL_M , the multivariate test for individual effect overall also revealed that gender (GLM test, $F=0.798$; $df=5$; $P=0.498$) had no significant effect on these parameters. (Table 1). The correlation between muscle total lipids and water content was highly significant ($R=-0.280$, $p<0.01$, $N=107$).

In order to investigate the existence of groups of samples with a similar NL fatty acid profile, a cluster analysis was done and revealed the existence of two groups. Group I comprised 59 individuals, most of them captured in the Minho (81.3% of the total sea lampreys captured in this river basin), the Vouga (100%) and the Tagus (100%), seven individuals from the Lima (38.9%) and one from the Mondego (5.3%) (Fig. 2). Group II comprised 51 individuals distributed as follows: 19 from the Douro, 18 from the Mondego, 11 from the Lima and 3 from the Minho, corresponding to 100%, 94.7%, 61.1% and 18.8% of the total of sea lampreys captured in each river basin, respectively (Fig. 2).

TABLE 2. – Relative amounts as a percentage of the sum (mean±sd) and frequency of occurrence (FO) of fatty acids in muscle neutral lipids of sea lamprey (*Petromyzon marinus*) from Cluster Groups I and II.

	Fatty Acids	Relative Amount Group I	FO (Group I)	Relative Amount Group II	FO (Group II)	
SFA	C10:0	0.04±0.01	98.0	0.05±0.02	100.0	
	C12:0	2.17±0.54	100.0	2.21±0.47	100.0	
	C13:0	0.77±0.44	100.0	0.96±0.48	100.0	
	C14:0	16.96±1.65	100.0	16.81±1.37	100.0	
	C15:0	0.04±0.01	100.0	0.05±0.04	88.2	
	C16:0	16.49±1.68	100.0	16.28±1.78	100.0	
	C17:0	0.02±0.01	98.3	0.05±0.02	62.8	
	C18:0	2.20±0.37	100.0	2.30±0.31	100.0	
	C20:0	0.16±0.08	98.3	0.16±0.05	100.0	
	C22:0	0.05±0.03	96.6	0.11±0.07	98.0	
	C23:0	0.03±0.02	18.6	0.13±0.12	60.8	
	C24:0	0.04±0.03	28.8	0.10±0.07	80.4	
	ΣSFA		38.9		39.2	
	MUFA	C14:1	0.93±0.28	100.0	0.94±0.26	100.0
C15:1		0.02±0.01	50.9	0.04±0.02	9.8	
C16:1		38.36±1.81	100.0	37.80±1.42	100.0	
C17:1		0.10±0.03	100.0	0.11±0.06	94.2	
C18:1ω9		16.78±1.59	100.0	17.34±1.31	100.0	
C20:1ω9		0.30±0.13	98.3	0.32±0.14	100.0	
C22:1ω9		0.06±0.05	100.0	0.10±0.06	86.3	
C24:1ω9		0.07±0.03	18.6	0.23±0.15	100.0	
ΣMUFA		56.6		56.8		
PUFA	C18:2ω6	0.15±0.09	100.0	0.28±0.17	100.0	
	C18:3ω6	0.02±0.03	57.6	0.06±0.05	72.9	
	C18:3ω3	0.04±0.02	96.6	0.08±0.05	96.7	
	C20:2ω6	0.11±0.09	100.0	0.20±0.14	39.2	
	C20:3ω6	0.06±0.06	100.0	0.08±0.05	100.0	
	C20:3ω3	0.56±0.59	100.0	0.52±0.17	100.0	
	C20:4ω6	0.07±0.09	83.1	0.14±0.12	92.2	
	C20:5ω3 (EPA)	0.70±0.28	100.0	0.86±0.26	100.0	
	C22:2ω6	0.05±0.04	84.8	0.10±0.07	49.0	
	C22:5ω3 (DPA)	0.70±0.27	100.0	0.85±0.28	100.0	
	C22:6ω3 (DHA)	1.20±0.58	100.0	1.33±0.53	100.0	
	ΣPUFA		3.7		4.3	
	Σ(PUFA+MUFA)	60.30		61.10		
	ΣPUFA/ΣSFA	0.09		0.11		
	Σω3	3.20		3.59		
	Σω6	0.45		0.65		
	Σω3/Σω6	8.70		6.40		
	C22:6ω3/C20:5ω3	1.71		1.56		

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

TABLE 3. – Results of pairwise analysis testing fatty acid muscle neutral lipid profile of *Petromyzon marinus* between Cluster Groups I and II.

Pairwise Groups	t	P (perm)	Perm
I, II	8.3793	0.001	998

t, t-statistic; P(perm), P-value (permutations); Perm, number of permutations.

TABLE 4. – Summary of the results from the SIMPER analysis for fatty acids of muscle neutral lipids of *Petromyzon marinus* between individuals of Cluster Groups I and II (Average dissimilarity between groups = 8.21).

Parameters	Contribution to dissimilarity (%)
C24:1 ω 9	7.81
C13:0	5.78
C22:6 ω 3 (DHA)	5.07
C20:2 ω 6	5.03
C16:0	4.60
C14:0	4.27
C18:1 ω 9	4.18
C24:0	3.96
C20:4 ω 6	3.76
C12:0	3.76
C20:3 ω 3	3.94
C23:0	3.68
C16:1	3.65
C20:5 ω 3 (EPA)	3.62
C22:5 ω 3 (DPA)	3.41
C14:1	3.05
C22:2 ω 6	3.05
C18:2 ω 6	3.04
C20:3 ω 3	2.95
C18:3 ω 6	2.92
C20:1 ω 9	2.52
C22:0	2.38
C18:0	2.32
C18:3 ω 3	2.16
C17:0	2.02

Thirty-one fatty acids were identified and compared between Group I and II. The relative amounts of fatty acid composition of neutral lipid of sea lampreys are presented in Table 2. The neutral lipid profile revealed that monounsaturated fatty acids (*MUFA*) are the most representative (Group I, 56.6%; Group II, 56.8%), followed by saturated fatty acids (*SFA*) (Group I, 38.9; Group II, 39.2%). Polyunsaturated fatty acids (*PUFA*) showed much lower relative amounts (Group I, 3.7%; Group II, 4.3%). The most representative *SFA* were C14:0 and C16:0 and the most representative *MUFA* were C16:1 and C18:1 ω 9. The most representative *PUFA* were ω 3 fatty acids, mainly C22:6 ω 3 (*DHA*), C20:5 ω 3 (*EPA*), C22:5 ω 3 (*DPA*) and C20:3 ω 3. Furthermore, our results demonstrated that the proportion of *DHA*+*EPA*+*DPA* was 70.4% and 70.8% of the total muscle *PUFA* for Groups I and II, respectively. The percentage content of *EPA* and *DHA* demonstrates the dominance of *DHA* over *EPA* (*DHA/EPA* ratio). The ω 3/ ω 6 ratio for Groups I and II were 8.7 and 6.4, respectively. The abundance of fatty acids from the ω 3 and ω 6 series and the abundance of odd-chain fatty acids are also presented in Table 2. The PERMANOVA results

showed that group factor exhibited significant differences ($P \leq 0.001$) in the NL_M fatty acid profile of sea lamprey (Table 3). SIMPER identified several fatty acids that contribute to overall differences (Table 4). The average similarity within group individuals was 92.87 and 93.12 for Group I and Group II, respectively, whereas between Group I and II the average dissimilarity was 8.21. SIMPER analysis revealed that 25 of 31 fatty acids that were analysed contributed to the dissimilarity between Groups I and II. The fatty acid with the highest percentage of contribution to dissimilarity was C24:1 ω 9 (7.81%), as opposed to the situation observed for C17:0 (2.02%) (Table 4).

DISCUSSION

The sea lamprey has important economic value in Portugal, Spain and France, where it is subjected to commercial exploitation. It is classified as 'vulnerable' in both Portugal and Spain (Cabral *et al.* 2005, Doadrio 2001), 'near threatened' in France (IUCN France *et al.* 2010), and 'least concern' according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Freyhof and Kottelat 2008) and the European Red List of Freshwater Fishes (Freyhof and Brooks 2011). It is curious, however, to note that this species is considered threatened in the European countries holding the main populations, namely France, Spain and Portugal (Mateus *et al.* 2012). The sea lamprey is also listed in the OSPAR convention list (Convention for the Protection of the Marine Environment of the North-East Atlantic) of threatened and/or declining species, in Annex III (protected fauna species) of the Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats) and in Annex II of the European Union Habitats Directive (92/43/EEC), which lists animal and plant species of interest to the European Community whose conservation requires the designation of Special Areas of Conservation (SACs) by the member states (Mateus *et al.* 2012).

The continental phase of the lamprey's life cycle is well known but very little information has been gathered during the oceanic phase. The available data is limited to the report of occasional captures of a few host species with lamprey scars, or occasionally still attached to the fish (Farmer 1980). Accidental captures are obviously more common in the oceanic shallower areas, were the bulk of commercial fisheries are performed, but this should not be taken as an indication of a preference for preys that live in the water column; it is probably a biased information because this type of sampling does not cover the entire bathymetric range of the potential feeding grounds. The anadromous sea lamprey is reported to attack a wide variety of marine organisms, as listed in Table 5. The only study concerning the sea lamprey distribution during the oceanic phase of the life cycle was done by Halliday (1991), with a limited record of 80 sea lampreys captured in the northwest Atlantic. This study indicated that almost

TABLE 5. – List of confirmed marine organisms parasitized by sea lamprey (*Petromyzon marinus*, L.).

Host taxon	Ecology	Reference
CHONDRICHTHYES		
Basking sharks (<i>Cetorhinus maximus</i> Gunnerus, 1765)	Pelagic-oceanic; oceanodromous	Beamish (1980)
Sandbar shark (<i>Carcharhinus plumbeus</i> Nardo, 1827)	demersal; oceanodromous	Jensen and Schwartz (1994)
Dusky sharks (<i>Carcharhinus obscurus</i> Lesueur, 1818)	demersal; oceanodromous	Jensen and Schwartz (1994)
Tiger sharks (<i>Galeocerdo cuvier</i> Péron and Lesueur, 1822)	demersal; oceanodromous	Jensen <i>et al.</i> (1998)
Greenland shark (<i>Somniosus microcephalus</i> Bloch and Schneider, 1801)	demersal	Gallant <i>et al.</i> (2006)
OSTEICHTHYES		
Twaite shad (<i>Alosa fallax</i> Lacepède, 1803)	pelagic-neritic; anadromous	Silva <i>et al.</i> (2013b)
American shad (<i>Alosa sapidissima</i> Wilson, 1811)	pelagic-neritic; anadromous	Beamish (1980)
Alewife (<i>Alosa pseudoharengus</i> Wilson, 1811)	pelagic-neritic; anadromous	Beamish (1980)
Blueback shad (<i>Alosa aestivalis</i> Mitchell, 1814)	pelagic-neritic; anadromous	Beamish (1980)
Atlantic menhaden (<i>Brevoortia tyrannus</i> Latrobe, 1802)	pelagic-neritic; oceanodromous	Mansuetti (1962)
Golden grey mullet (<i>Liza aurata</i> Risso, 1810)	pelagic-neritic	Silva <i>et al.</i> (2013b)
Atlantic cod (<i>Gadus morhua</i> L., 1758)	demersal; oceanodromous	Beamish (1980)
Atlantic herring (<i>Clupea harengus</i> , L., 1758)	demersal; oceanodromous	Farmer (1980)
Haddock (<i>Melanogrammus aeglefinus</i> L., 1758)	demersal; oceanodromous	Beamish (1980)
Saithe (<i>Pollachius virens</i> L., 1758)	demersal; oceanodromous	Beamish (1980)
Red hake (<i>Urophycis chuss</i> Walbaum, 1792)	demersal; oceanodromous	Farmer (1980)
Atlantic mackerel (<i>Scomber scombrus</i> L., 1758)	pelagic-neritic; oceanodromous	Farmer (1980)
Atlantic salmon (<i>Salmo salar</i> L., 1758)	demersal; anadromous	Beamish (1980); Silva <i>et al.</i> (2013b)
Sea trout (<i>Salmo trutta</i> L., 1758)	pelagic-neritic; anadromous	Silva <i>et al.</i> (2013b)
Brook trout (<i>Salvelinus fontinalis</i> Mitchell, 1814)	demersal; anadromous	Beamish (1980)
Swordfish (<i>Xiphias gladius</i> L., 1758)	pelagic-oceanic; oceanodromous	Beamish (1980)
Striped bass (<i>Morone saxatilis</i> Walbaum, 1792)	demersal; anadromous	Beamish (1980)
Bluefish (<i>Pomatomus saltatrix</i> L., 1758)	pelagic-oceanic; oceanodromous	Beamish (1980)
Squeteague (<i>Cynoscion regalis</i> Bloch and Schneider, 1801)	demersal; oceanodromous	Beamish (1980)
CETACEA		
Fin whales (<i>Balaenoptera physalus</i> L., 1758)	Pelagic-oceanic	Japha (1910)
Sei whale (<i>Balaenoptera borealis</i> Lesson, 1828)	Pelagic-oceanic	Japha (1910)
Harbour porpoise (<i>Phocoena phocoena</i> L., 1758)	Pelagic-oceanic	van Utrecht (1959)
Western North Atlantic right whale, (<i>Eubalaena glacialis</i> Müller, 1776)	Pelagic-oceanic	Nichols and Hamilton (2004)
Minke whale (<i>Balaenoptera acutorostrata</i> Lacépède, 1804)	Pelagic-oceanic	Nichols and Tschertter (2011)
Killer whale (<i>Orcinus orca</i> L.)	Pelagic-oceanic	Samarra <i>et al.</i> (2012)

all individuals less than 39 cm long were taken in bottom trawls on the continental shelf or in coastal trap nets, whereas most animals more than 56 cm long were captured in mid-water trawls along the shelf edge or on the continental slope (Halliday 1991). According to Haedrich (1977), it is even quite possible that sea lamprey may often feed in deeper offshore waters as they have been caught at considerable depths: as deep as 4099 m. It is interesting to note that sea lamprey and Pacific lamprey (*Entosphenus tridentatus* Gairdner in Richardson, 1836) seem to have in common the fact that they both prey upon demersal and pelagic hosts, and that they can be captured by trawls in benthic or pelagic environments.

A study by Orlov *et al.* (2008) aimed at better understanding the ecology of the parasitic life stage of the Pacific lamprey in the North Pacific is probably the most comprehensive study related to feeding ecology and spatial distribution of an anadromous lamprey species in the marine environment. By clustering the data on long-term bottom and mid-water trawl-recorded captures, Orlov *et al.* (2008) noticed that the average catches and frequency of occurrence of Pacific lamprey decreased with an increase in depth. In bottom trawl surveys, Pacific lamprey was found at a maximum depth of 1163 m, and in mid-water trawls as deep as 1485 m. The same study identified 10 preferred host species for the Pacific lamprey composed

of demersal and pelagic groups of species such as flatfishes (4 species: Greenland halibut *Reinhardtius hippoglossoides* Walbaum, 1792; Pacific halibut *Hippoglossus stenolepis* Schmidt, 1904; Arrow-tooth flounder *Atheresthes stomias* Jordan and Gilbert, 1880; and Kamchatka flounder *Atheresthes evermanni* Jordan and Starks, 1904), salmon (3 species: Chum salmon *Oncorhynchus keta* Walbaum, 1792; sockeye salmon *Oncorhynchus nerka* Walbaum, 1792; and Chinook salmon *Oncorhynchus tshawytscha* Walbaum, 1792), codfish (2 species: Walleye Pollock *Theragra chalcogramma* Pallas, 1814; and Pacific cod *Gadus macrocephalus* Tilesius, 1810), and the Pacific herring (*Clupea pallasii pallasii* Valenciennes, 1847).

Skeletal muscle is an important fat reserve in the beginning of the sea lamprey spawning migration (Lança *et al.* 2011), representing in our study 21%-28% of the total muscle dry weight and 52%-79% of muscle total lipids. In fact, this is an empirical knowledge shared by professional fisherman and local consumers, who prefer lampreys caught in freshwater, in upstream reaches, after spending part of this fat reserve in their upstream migration. This fact was recently described for the River Minho's sea lampreys, for which there was a significant reduction in the amount of lipids in the muscle of those caught 65 km upstream from the sea in comparison with those caught at the river mouth (Araújo 2011).

Triacylglycerols in fish are stored in various tissues, including muscle, liver, sub-dermal tissue and mesenteries and during starvation they are mobilized preferentially for basic maintenance and other metabolic needs (Adams 1999). The same occurs in lampreys, since they store large amounts of triacylglycerols in liver and body wall muscles (Plisetskaya 1980). The muscle fatty acid profile of the individuals from Groups I and II displays similarities, especially in terms of *MUFA*, which are the major fatty acids in neutral lipids, followed by *SFA* and *PUFA*. These results are also in agreement with the common opinion that fish species accumulate depot lipids composed mainly of *SFA* and *MUFA* (Kozlova and Khotimchenko 2000, Pinela *et al.* 2009). The major *SFA* were C14:0 and C16:0 and the major *MUFA* were C16:1 and C18:1 ω 9c and sea lampreys showed a normal pattern for marine fish lipids, where *EPA* and *DHA* are dominant *PUFA* and ω 3/ ω 6 ratios showed a typical value that usually ranges from 4.7 to 14.4 (Henderson and Tocher 1987).

Our results revealed that 25 of the 31 fatty acids analysed contributed to the dissimilarity of muscle neutral lipid profile between Groups I and II, which raises the possibility of differences in sea lamprey preferential food sources and/or feeding strategies. The dissimilarity between the two groups was supported by five main differences related to the following fatty acids:

(i) *Odd-chain fatty acids* (C13:0, C17:0, C23:0). Several odd fatty acids are originally derived from bacteria and are considered useful markers of microbial presence on detritus (Lechevalier 1982, Mayzaud *et al.* 1989, Kaneda 1991, Rajendran *et al.* 1993, Napolitano 1999), especially the C15:0; C15iso; C15anteiso; C17:0; C17iso and C17anteiso fatty acids. What is very interesting to note is that individuals of Group II revealed the higher amounts for all three odd-chain fatty acids, which probably means that sea lampreys fed upon a dietary source rich in these fatty acids, like fish that include in their diet decaying matter or plant debris, namely the mugilidae (Almeida *et al.* 1993), or scavenger benthic species that feed on discards from commercial fishing vessels (Dunn *et al.* 2010).

(ii) *Saturated fatty acids* (C12:0, C14:0, C16:0, C18:0, C20:0 and C24:0) From these, we excluded short and medium chain fatty acids (i.e. C12:0, C14:0) and C16:0 and C18:0. The former are not useful markers in diet studies because they are almost oxidized before deposition in animal tissues, and when present in tissues they have arisen from *de novo* biosynthesis (Tocher 2003, Budge *et al.* 2006). C16:0 and C18:0 were eliminated because, although vertebrates synthesize few fatty acids, these tend to be restricted to C14:0, C16:0 and C18:0, which make them ubiquitous. Our attention was focused on C24:0. Long-chain saturated fatty acids (from C24:0 to C28:0) in the marine environment generally stem from higher plant waxes and are more abundant in deep than in surface sediments near the shore (Rielley *et al.* 1991, Muri *et al.*

2004). Group II is once again the group with the highest values of C24:0 (relative amount and frequency of occurrence), which raises the hypothesis that sea lampreys have a feeding strategy relatively close to the continent.

(iii) *Fatty acids of ω -3-family* (C18:3 ω 3; C20:3 ω 3, C20:5 ω 3; C22:5 ω 3; C22:6 ω 3). C20:5 ω 3 (diatoms) and C22:6 ω 3 (dinoflagellates) are important phytoplankton markers (Napolitano 1999, Dalsgaard *et al.* 2003). In fact, algae are the only organisms that possess the enzymes for C20:5 ω 3 and C22:6 ω 3 production (Iverson 2009). Apart from the statistical differences, this result only reveals a typical scenario of a marine food web with a planktonic base for both groups.

(iv) *Fatty acids of ω -6-family* (C18:2 ω 6, C18:3 ω 6, C20:2 ω 6, C20:4 ω 6 and C22:2 ω 6). These fatty acids are possible markers of benthic algae (Sargent and Whittle 1981) and recognized markers of freshwater fish (Bell *et al.* 1986, Henderson and Tocher 1987, Käkälä, *et al.* 1993). Although present in both groups, the sea lampreys from Group II showed a tendency to have greater amounts of these fatty acids in their tissue, which indicates a diet with a more continental profile, i.e. fish species that belong to a food web with a strong input from estuarine environments;

(v) *C24:1 ω 9*. This fatty acid is ubiquitous in the marine environment and is found at a few tenths of a percent in most marine organisms, including practically all marine fish. Only 18.6% of the animals from Group I had this fatty acid in their tissues, whereas it was present in all the animals from Group II in higher amounts than those found in Group I. Since this fatty acid was first described in elasmobranchs, it was first believed to be characteristic of fats of elasmobranch fish (original name: selacholeic acid) (Laposata 1998). However, based only on C24:1 ω 9, it is not possible to stress the importance of elasmobranchs in the feeding strategy of sea lampreys.

With respect to C18:1 ω 9, C20:1 ω 9 and C22:1 ω 9 fatty acids, our results also revealed significant differences. Generally when found in higher trophic level organisms, C20:1 ω 9 and C22:1 ω 9 are thought to originate from copepods and they are also considered good trophic markers of zooplankton (herbivorous calanoid copepods) (Dalsgaard *et al.* 2003), whereas C18:1 ω 9 is considered an important biomarker for carnivory. Investigations with marine fish showed that C20:1 ω 9 is particularly abundant in many fish triacylglycerols and is derived from the corresponding C20:1 ω 9 fatty alcohol in zooplanktonic wax esters (Ackman 1999). These results suggest that both feeding strategies may include hosts that have consumed copepods directly or consumed copepod consumers.

Host attachment and scarring data confirm that sea lamprey in the northwest Atlantic prey upon many species in at least three trophic levels (Table 5). Drevnick *et al.* (2006) corroborate this with a stable isotope analyses to assess the sea lamprey diet and confirm that anadromous sea lamprey do not feed at a single

trophic level during the adult stage. According to these authors, the individuals sampled appeared to have fed on fish from both benthic and pelagic habitats.

In our opinion and based on our results, the sea lamprey clustered in Group II may have had a feeding strategy that promoted parasitism on demersal fish from the mesopelagic or even bathypelagic zone. This assumption is supported by two evidences. First, there are no catch data of lampreys attached to hosts (or free swimming), or records of scarred fish, in the bottom trawl fishery on the Portuguese continental shelf (unpublished data). Second, higher relative amounts of odd-chain fatty acids, especially C17:0, higher relative amounts of fatty acids of ω -6 series, and the presence of the long-chain fatty acid C24:0 in NL profile of sea lampreys from Group II, might indicate a feeding strategy that includes a diet with a more continental profile: fish species that have fed upon resources originating in estuarine environment.

In summary, based on our results, we have reasons to believe that it is possible to distinguish two feeding strategies in sea lampreys from the west coast of Portugal. Group I (i.e. Rivers Minho, Tagus, Vouga and ca. 39% of the Lima) showed a feeding strategy that resembles a typical marine food web with a planktonic support. On the other hand, Group II (Douro, Mondego and ca. 61% of the Lima) showed a much more diverse feeding strategy, including the marine food web with a planktonic support described for Group I, together with biochemical clues that indicate that at least during part of their parasitic trophic phase these animals fed upon benthic opportunist carnivorous/scavenger fish species, probably elasmobranchs, and teleosts from a food web with a strong detritus and benthic algae signal. Regarding this last remark, we should be aware that in Portugal and Galicia it is common to observe sea lamprey juveniles feeding upon mugilids (Mugilidae) near the mouth of some estuaries between November and May (unpublished data; Silva *et al.* 2013b). This could explain the evidence of parasitic feeding on detritivorous species found in sea lampreys from Group II. The importance of this initial parasitic feeding stage becomes even more significant if we bear in mind that some of these animals stay no more than 14 months at sea. In a recent study, researchers from the University of Santiago de Compostela tagged and released sea lamprey juveniles that were already feeding upon mugilids. In the following year they recaptured one of the animals, which had increased its length from 21.8 cm to 89.5 cm, and its mass from 20 g to 1218 g (Silva *et al.* 2013a).

The information concerning the feeding ecology of the parasitic stage of the sea lamprey may be particularly useful to support management actions aimed at conserving this threatened species. A hypothesis that we will try to test in the future is the possibility that these feeding strategies are associated with different dispersion tactics of this species during the parasitic stage. In fact, sea lamprey individuals that prefer-

entially parasitize pelagic hosts can be engaged in a strategy that enhances dispersion but also increases the risk of not returning to continental waters to reproduce, versus a more conservative strategy with animals feeding mainly on demersal hosts with limited mobility and associated with the continental slope or even the ocean basin floor.

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